Amendments to the Specification:

At page 1, line 1, please replace the paragraph setting forth the title ("Peptide"), with the following paragraph:

-- ANALOGS OF GASTRIC INHIBITORY POLYPEPTIDE AND THEIR USE FOR TREATMENT OF DIABETES --

Please add the following new paragraphs at page 1, between lines 2 and 3:

-- CROSS REFERENCE TO RELATED APPLICATIONS

This application is a U.S. National Phase Application of International Application No. PCT/GB00/01089, which designated the United States and was filed on March 29, 2000 and published in English, which in turn claims the benefit of GB9907216.7, filed on March 29, 1999, and GB99917565.5, filed July 27, 1999. The entire teachings of the above applications are incorporated herein by reference. --

Please replace the first two paragraphs on page 5 with the following paragraphs:

~~Fig. 1.~~ Primary structure of human gastric inhibitory polypeptide (GIP) (SEQ ID NO:1)

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     1              5                  10                 15
NH2-Tyr-Ala-Glu-Gly-Thr-Phe-Ile-Ser-Asp-Tyr-Ser-Ile-Ala-Met-Asp-
                   20                  25                 30
    Lys-Ile-His-Gln-Gln-Asp-Phe-Val-Asn-Trp-Leu-Leu-Ala-Gln-Lys-
                   35                 40
    Gly-Lys-Lys-Asn-Asp-Trp-Lys-His-Asn-Ile-Thr-Gln-COOH
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~~Fig. 2.~~ Primary structure of porcine gastric inhibitory polypeptide (GIP) (SEQ ID NO:2)

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     1               5                  10                  15
NH2-Tyr-Ala-Glu-Gly-Thr-Phe-Ile-Ser-Asp-Tyr-Ser-Ile-Ala-Met-Asp-
                     20                  25                  30
    Lys-Ile-Arg-Gln-Gln-Asp-Phe-Val-Asn-Trp-Leu-Leu-Ala-Gln-Lys-
                     35                  40
    Gly-Lys-Lys-Ser-Asp-Trp-Lys-His-Asn-Ile-Thr-Gln-COOH
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Please replace the paragraph running from page 5, line 20, through page 6, line 2, with the following paragraph, as N-acetylated GIP and N-acetyl-GIP are redundant:

> Amino-terminally modified GIP analogues include N-glycated GIP(1-42), N-alkylated GIP(1-42), N-acetylated ~~N-actylated~~ GIP(1-42)~~, N-acetyl-GIP(1-42)~~ and N-isopropyl GIP(1-42).

Please replace the paragraph on page 6, line 31, with the following paragraph:

> (l) conversion of Ala^2-Glu^3 bond to a psi [CH_2NH] ~~[CH2NH]~~ bond

Please replace the paragraph on page 7, lines 1-2, with the following paragraph:

> (m) conversion of Ala^2-Glu^3 ~~Ala2-Glu3~~ bond to a stable peptide isostere ~~isotere~~ bond

Please replace the paragraph running from page 12, line 6 to page 13, line 5 with the following paragraph:

The N-terminal modification of GIP is essentially a three step process. Firstly, GIP is synthesized from its C-terminus ~~C-terminal~~ (starting from a Fmoc-Gln (Trt)-Wang resin, Novabiochem) up to the penultimate N-terminal amino-acid (Ala^2) ~~(Ala2)~~ on an automated peptide synthesizer (Applied Biosystems, CA, USA). The synthesis follows standard Fmoc peptide chemistry protocols. Secondly, the N-terminal amino-acid of native GIP (Tyr) is added to a manual bubbler system as a Fmoc-protected Tyr(tBu)-Wang resin. This amino acid is deprotected at its N-terminus (piperidine in DMF (20% v/v)) and allowed to react with a high concentration of glucose (glycation, under reducing conditions with sodium cyanoborohydride), acetic anhydride (acetylation), pyroglutamic acid (pyroglutamyl) etc. for up to 24 h as necessary to allow the reaction to go to completion. The completeness of reaction will be monitored using the ninhydrin test which will determine the presence of available free α-amino ~~a-amino~~ groups. Thirdly, (once the reaction is complete) the now structurally modified Tyr is cleaved from the wang resin (95% TFA, and 5% of the appropriate scavengers. N.B. Tyr is considered to be a problematic amino acid and may need special consideration) and the required amount of N-terminally modified-Tyr consequently added directly to the automated peptide synthesizer, which will carry on the synthesis, thereby ~~therby~~ stitching the N-terminally modified-Tyr to the α-amino ~~a-amino~~ of GIP(Ala^2) ~~(Ala2)~~, completing the synthesis of the GIP analogue. This peptide is cleaved off the Wang resin (as above) and then worked up using the standard Buchner filtering, precipitation ~~precipation~~, rotary evaporation and drying techniques.

Please replace the paragraph on page 16, lines 4-24, with the following paragraph:

Electrospray ionization mass spectrometry (ESI-MS). Samples for ESI-MS analysis containing intact and degradation fragments of GIP (from DPP IV and plasma incubations) as well as Tyr^1-glucitol GIP, were further purified by HPLC. Peptides were dissolved (approximately 400 pmol) in 100 μl of water and applied to the LCQ benchtop mass spectrometer (Finnigan MAT, Hemel Hempstead, UK) equipped with a microbore C-18 HPLC column (150 x 2.0mm, Phenomenex, UK, Ltd, Macclesfield). Samples (30μl direct loop injection) were injected at a flow rate of 0.2ml/min, under isocratic conditions 35% (v/v) acetonitrile/water ~~acetonitile/water~~. Mass spectra were obtained from the quadripole ion trap mass analyzer and recorded. Spectra were collected using full ion scan mode over the mass-to-charge (m/z) range 150-2000. The molecular masses of GIP and related structures were determined from ESI-MS profiles using prominent multiple charged ions and the following equation $M_r = iM_i$ (where M_r = molecular mass; M_i = m/z ratio; i = number of charges; M_h = mass of a proton).

Please replace the paragraph running from page 16, line 26 to page 17, line 16, with the following paragraph:

***In vivo* biological activity of GIP and Tyr^1-glucitol ~~Try1-glucitol~~ GIP.** Effects of GIP and Tyr^1-glucitol GIP on plasma glucose and insulin concentrations were examined using 10-12 week old male Wistar rats. The animals were housed individually in an air conditioned room and 22±2°C with a 12 hour light/12 hour dark cycle. Drinking water and a standard rodent maintenance diet (Trouw Nutrition, Cheshire, UK ~~Belfast~~) were supplied *ad libitum*. Food was withdrawn ~~withdrawm~~ for an 18 hour period prior to intraperitoneal injection of glucose alone (18mmol/kg body weight) or in combination with either GIP or Tyr^1-glucitol GIP (10 nmol/kg). Test solutions were administered in a final volume of

8 ml/kg body weight. Blood samples were collected at 0, 15, 30 and 60 minutes from the cut tip of the tail of conscious rats into chilled fluoride/heparin microcentrifuge tubes (Sarstedt, Nümbrecht, Germany). Samples were centrifuged using a Beckman microcentrifuge for about 30 seconds at 13,000 g. Plasma samples were aliquoted and stored at -20°C prior to glucose and insulin determinations. All animal studies were done in accordance with the Animals (Scientific Procedures) Act 1986.

Please replace the paragraph running from page 18, line 18 to page 19, line 5, with the following paragraph:

Degradation of GIP and Tyr^1-glucitol GIP by human plasma. Figure 2 shows a set of typical HPLC profiles of the products obtained from the incubation of GIP or Tyr^1-glucitol GIP with human plasma for 0 and 4 h. GIP (Fig 2a) with a retention time of 22.06 min was readily metabolised by plasma within 4 hours incubation giving rise to the appearance of a major degradation peak with a retention time of 21.74 minutes. In contrast, the incubation of Tyr^1-glucitol GIP under similar conditions (Fig 2b) did not result in the formation of any detectable degradation fragments during this time with only a single peak being observed with a retention time of 21.77 minutes. Addition of diprotin A, a specific inhibitor of DPP IV, to GIP during the 4 hours incubation completely inhibited degradation of the peptide by plasma. Peaks corresponding with intact GIP, GIP (3-42) and Tyr^1-glucitol GIP are indicated. A major peak corresponding to the specific DPP IV inhibitor tripeptide DPA appears in the bottom panels ~~peanels~~ with retention time of 16.29 ~~16-29~~ min.

Please replace the paragraph running from page 21, line 13 to page 22, line 3, with the following paragraph:

Compared with the control group, plasma glucose concentrations and area under the curve (AUC) were significantly lower following administration of either GIP or Tyr^1-glucitol GIP (Figure 4A, B). Furthermore, individual values at 15 and 30 minutes together with AUC were significantly lower following administration of Tyr^1-glucitol GIP as compared to GIP. Consistent with the established insulin-releasing properties of GIP, plasma insulin concentrations of both peptide-treated groups were significantly raised at 15 and 30 minutes compared with the values after administration of glucose alone (Figure 5A). The overall insulin responses, estimated as AUC were also <u>significantly</u> ~~signigicantly~~ greater for the two peptide-treated groups (Figure 5B). Despite lower prevailing glucose concentrations than the GIP-treated group, plasma insulin response, calculated as AUC, following Tyr^1-glucitol GIP was significantly greater than after GIP (Figure 5B). The significant elevation of plasma insulin at 30 minutes is of particular note, suggesting that the insulin-releasing action of Tyr^1-glucitol GIP is more protracted than GIP even in the face of a diminished glycemic stimulus (Figures 4A, 5A).

Please replace the paragraph on page 22, lines 7-23, with the following paragraph:

The forty two amino acid GIP is an important incretin hormone released into the circulation from endocrine K-cells of the duodenum and jejunum following ingestion of <u>food.</u> ~~food .~~ The high degree of structural conservation of GIP among species supports the view that this peptide plays and important role in metabolism. Secretion of GIP is <u>stimulated</u> ~~stimulateed~~ directly by actively transported nutrients in the gut lumen without a notable input from automatic

nerves. The most important stimulants of GIP release are simple sugars and unsaturated long chain fatty acids, with amino acids exerting weaker effects. As with tGLP-1, the insulin-releasing effect of GIP is strictly glucose-dependent. This affords protection against hypoglycemia and thereby fulfills ~~fulfils~~ one of the most desirable features of any current or potentially new antidiabetic drug.

Please replace the paragraph running from page 25, line 21 to page 26, line 3, with the following paragraph:

In conclusion, this study has demonstrated for the first time that the glycation of GIP at the amino-terminal Tyr^1 residue limits GIP catabolism through impairment of the proteolytic actions of serum peptidases ~~petidases~~ and thus prolongs its half-life *in vivo*. This effect is accompanied by enhanced antihyperglycemic activity and raised insulin concentrations *in vivo*, suggesting that such DPP IV resistant analogues should be explored alongside tGLP-1 as potentially useful therapeutic agents for NIDDM. Tyr^1-glucitol GIP appears to be particularly interesting in this regard since such amino-terminal modification of GIP enhances rather than impairs glucose-dependent insulinotropic potency as was observed recently for tGLP-1.

Please replace the paragraph running from page 26, line 20 to page 27, line 2, with the following paragraph:

High-performance liquid chromatography (HPLC) grade acetonitrile was obtained from Rathburn (Walkersburn, Scotland). Sequencing grade trifluroacetic acid (TFA) was obtained from Aldrich (Poole, Dorset, UK). Dipeptidyl peptidase IV was purchased from Sigma (Poole, Dorset, UK), and Diprotin A was purchased

from Calibiochem Novabiochem (Beeston, Nottingham, UK). RPMI 1640 tissue culture medium, fetal ~~foetal~~ calf serum, penicillin and streptomycin were all purchased from Gibco (Paisley, Strathclyde UK). All water used in these experiments was purified using a Milli-Q, Water Purification System (Millipore, Milford, ~~Millford,~~ MA, USA). All other chemicals used were of the highest purity available.

Please replace the paragraph on page 28, lines 14-29, with the following paragraph:

Before experimentation, the cells were harvested from the surface of the tissue culture flasks with the aid of trypsin/EDTA (Gibco, Paisley, Strathclyde, UK), seeded into 24-multiwell plates (Nunc, Roskilde, Denmark) at a density of 1.5 x 10^5 ~~105~~ cells per well, and allowed to attach overnight at 37°C. Acute tests for insulin release were preceded by 40 min pre-incubation at 37°C in 1.0 ml Krebs Ringer bicarbonate buffer (115 mM NaCl, 4.7 mM KCl, 1.28 mM $CaCl_2$, 1.2 mM KH_2PO_4, 1.2 mM $MgSO_4$, 10 mM $NaHCO_3$, 5 g/l bovine serum albumin, pH 7.4) supplemented with 1.1 mM glucose. Test incubations were performed (n=12) at two glucose concentrations (5.6 mM and 16.7 mM) with a range of concentrations (10^{-13} to 10^{-8} M) of GIP or GIP analogues. After 20 min incubation, the buffer was removed from each well and aliquots (200 μl) were used for measurement of insulin by radioimmunoassay [31].

Please replace the paragraph on page 29, lines 11-16, with the following paragraph:

The monoisotopic molecular masses of the peptides were determined using ESI-MS. After spectral averaging was performed, prominent multiple charged species $(M+3H)^{3+}$ ~~(M+3H)3+~~ and $(M+4H)^{4+}$ ~~(M+4H)4+~~ were detected for each peptide.

Calculated molecular masses confirmed the structural identity of synthetic GIP and each of the N-terminal analogues.